RESEARCH PLAN PROPOSAL

Influence of intake of Pumpkin seeds extract in the male reproductive toxicity caused by antidepressant drug; Escitalopram in *Swiss albino* mice

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INTRODUCTION

DEPRESSION

Hectic life style, lack of exercise and lack of healthy diet which are more common now a days is a reason for depression. Depression is the common mental disorder that presents with depressed mood, loss of interest or pleasure, feeling of guilt or low self- worth, disturbed sleep or appetite, low energy(Ilgin *et al*,2018).
According to the Centers for Disease Control and Prevention (CDC), 7.6 percent of people over the age of 12 have depression in any 2-week period. This is substantial and shows the scale of the issue. According to the World Health Organization (WHO), depression is the most common illness worldwide and the leading cause of disability. They estimate that 350 million people are affected by depression, globally (Ilgin *et al*,2018).

Signs and symptoms

Symptoms of depression can include:

- depressed mood
- reduced interest or pleasure in activities previously enjoyed, loss of sexual desire
- unintentional weight loss (without dieting) or low appetite
- insomnia (difficulty sleeping) or hypersomnia (excessive sleeping)
- psychomotor agitation, for example, restlessness, pacing up and down
- delayed psychomotor skills, for example, slowed movement and speech
- fatigue or loss of energy
- feelings of worthlessness or guilt
- impaired ability to think, concentrate, or make decisions
- recurrent thoughts of death or suicide, or attempt at suicide.

Causes

The causes of depression are not fully understood and may not be down to a single source. Depression is likely to be due to a complex combination of factors that include:

- genetics
- biological changes in neurotransmitter levels
- environmental
- psychological and social (psychosocial)

Some people are at higher risk of depression than others; risk factors include:

- Life events: These include bereavement, divorce, work issues, relationships with friends and family, financial problems, medical concerns or acute stress.
- Personality: Those with less successful coping strategies or previous life trauma are more susceptible.
- Genetic factors: Having a first-degree relatives with depression increases the risk.
- Childhood trauma.
- Some prescription drugs: These include corticosteroids, some beta-blockers, interferon, and other prescription drugs.
- Abuse of recreational drugs: Abuse of alcohol, amphetamines and other drugs are strongly linked to depression.
- A past head injury.
- Having one episode of major depression: This increases the risk of a subsequent one.
- Chronic pain syndromes: These and other chronic conditions, such as diabetes, chronic obstructive pulmonary disease, and cardiovascular disease make depression more likely.

ANTIDEPRESSANTS

Drug which enhance the alterness and may result in an increased output of behaviour. Antidepressants are a class of drugs that reduce symptoms of depressive disorders by correcting chemical imbalances of neurotransmitters in the brain. Chemical imbalances may be responsible for changes in mood and behavior. Antidepressant drugs work against psychotic disorders such as depression or generalized anxiety and mood disorders.(Bandegi *et al*,2018)

TYPES OF ANTIDEPRESSANTS

Selective Serotonin Reuptake Inhibitors (SSRIs)

These are among the newer antidepressants, which have been available from 1988. They act on the neurotransmitter (brain chemical) serotonin. Some of the trade names in this class include Aropax (paroxetine), Prozac, Lorien, Nuzak, Lily-Fluoxetine (fluoxetine), Cipramil (citalopram), Cipralex (escitalopram), Zoloft (sertraline). Luvox (fluvoxamine) and Brintellix (vortioxetine). (Atli *et al*,2017)

This group of drugs, together with the other newer agents, is the most widely prescribed due to the favourable side-effect profile and relative safety if taken in overdose. Different drugs in this class are also registered for treatment of anxiety disorders, panic disorders, post-traumatic stress disorders, obsessive-compulsive disorder and social phobia. Side effects may be present during the first few weeks of therapy, but usually disappear after a while. These are often diminished by starting medication in low dosages and gradually increasing until a therapeutic dosage is reached (Ilgin *et al* ,2017)

Common side effects include:

- Nausea (take after food)
- Headache (improves after a while; start with low dosages)
- Agitation/anxiety
- Sleep disturbances
- Decreased appetite
- Sexual disturbances

Serotonin and Noradrenaline Reuptake Inhibitors (SNRIs)

This class of medications is closely related to the SSRIs, but have an additional mechanism of action in that they also affect noradrenaline reuptake. Medications in this class include venlafaxine (Efexor, Venlor), duloxetine (Cymbalta) and Pristiq (desvenlafaxine).

There is some evidence that this class of medications may be more effective in preventing relapse episodes of depression. They are also used when the depression

is accompanied by painful physical symptoms such as headaches and muscle pain. Their side-effect profile is similar to that of the SSRIs.

Tricyclics

This is an older group of drugs, which has been in use since 1957. These drugs affect predominantly noradrenaline. Some of the drugs in this class include Tryptanol, Trepiline (amitriptyline), Tofranil, Ethipramine (imipramine), Anafranil (clomipramine), Emdalen (lofepramine), Aventyl (nortriptyline) and others.

Tricyclics are also used for the treatment of anxiety disorders, sleep disorders, pain relief, migraine prophylaxis and bedwetting (imipramine). Some patients, particularly the elderly, find the side effects of these drugs more difficult to tolerate. Tricyclics are not safe in overdose, and in the event of more tablets being taken than prescribed, it's important to seek medical advice urgently. Despite the side-effect profile, tricyclics are extremely effective antidepressants.

Monoamine Oxidase Inhibitors (MAOIs)

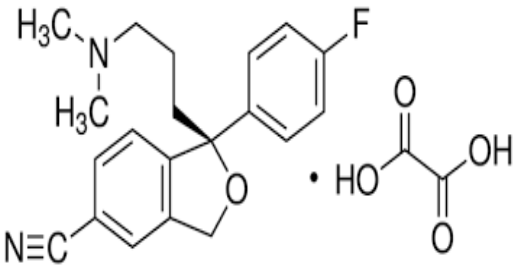
This is an older group of antidepressants, which is used less frequently today.These agents act by inhibiting an enzyme called monoamine oxidase, which usually breaks down serotonin, noradrenaline and dopamine in the brain. This results in an increase in these neurotransmitters, the deficiency of which is associated with depressive illness. However, certain foodstuffs containing tyramine (e.g. cheese, red wine, processed meats and many others) also require monoamine oxidase for their metabolism. The inhibition of this enzyme results in an excess of tyramine, which acts upon the blood vessels to cause a rise in blood pressure. This rise may sometimes be fatal, which is why patients taking MAOIs need to observe dietary restrictions. The danger of any food or drug reaction persists for about 14 days after stopping treatment with a MAOI. A washout period is therefore required before starting a different antidepressant. Medications include: isocarboxazid (Marplan), nialamide (Niamid), phenelzine (Nardil, Nardelzine), tranylcypromine (Parnate, Jatrosom), rasagiline (Azilect), selegiline (Deprenyl) and eldepryl (Emsam). Some MAOIs are available that don't completely inhibit the monoamine oxidase enzyme and dietary restrictions, like moclobemide (Aurorix, Manerix)

MAOIs are thought to be particularly useful in treating depression. They are also useful when depression is not responding to other drugs and in phobia and panic disorder.

ESCITALOPRAM OXALATE (ANTIDEPRESSANT DRUG)

- Escitalopram, the *S*-enantiomer of citalopram, belongs to a class of antidepressant agents known as selective serotonin-reuptake inhibitors (SSRIs) which specifically binds with serotonin transporter(SERT).
- Lexapro tablets are film-coated, round tablets containing escitalopram oxalate in strengths equivalent to 5 mg, 10 mg, and 20 mg escitalopram base. Lexapro (escitalopram oxalate) is available as tablets or as an oral solution.

Escitalopram oxalate is designated S-(+)-1-[3(dimethyl-amino)propyl]-1-(p-fluorophenyl)-5-phthalancarbonitrile oxalate with the following structural formula:



The molecular formula is $C_{20}H_{21}FN_2O \bullet C_2H_2O_4$ and the molecular weight is 414.40.The recommended dose of Lexapro is 10 mg or 20 mg/ day.

Common side effects include trouble sleeping, nausea, sexual problems and feeling tired.

In August 2002, the FDA approved Lexapro for the treatment of major depressive disorder. In 2016 it was the 26th most prescribed medication in the United States with more than 25 million prescriptions.

PUMPKIN (*Cucurbita maxima*) SEEDS

Pumpkin belongs to the family of Cucurbitaceae. The pumpkin plant along with its seeds has been used in the traditional medicine in many countries, including India.

Pumpkin contains calcium, iron, vitamin A, oil (25-55%, rich in unsaturated oleic and linoleic acids), protein (25-35%) with high amounts of arginine, aspartate and glutamic acid, although it is deficient in lysine and sulphur containing amino acids. The chemical constituents from seeds contain 30% unsaturated fatty oil (linoleic and oleic fatty acids). Triterpenoids, flavonoids, coumarins, saponines, cucurbitacins, antioxidant vitamins such as tocopherol and carotenoids, minerals, trace elements such as zinc and selenium, amino acid are also found in Pumpkin seeds. The highly unsaturated fatty acid composition of pumpkin seed makes it well-suited for improving nutritional benefits from foods. Due to the L-tryptophan content of pumpkin seeds, they have been suggested to help remedy depression. Tryptophan is an essential amino acid present in pumpkin seed, and 5-hydroxytryptophan (5-HTP) is the intermediate metabolite of tryptophan in the formation of the neurotransmitter serotonin. Both tryptophan and 5-HTP are promoted as treatment for depression. (George *et al*,2012).

Pumpkin seed extract is useful for immunomodulation, reproductive health and therapeutic advantage over a wide range of disease conditions. The antioxidative properties of pumpkin seed extract could also improve fertility and it helps to prevent arteriosclerosis, high blood pressure and heart diseases. (Aghaie *et al*, 2016).

Pumpkin seeds improve libido and semen quality by elevating the serum testosterone level .It has been demonstrated that pumpkin seeds and daily rich diet of zinc can decrease the undesirable side effect of lead contaminants and improve the sexual health status . Also, pumpkin seed can inhibit citral induced hyperplasia of the ventral prostate lobe by reducing protein binding prostate levels (PBP) , weight of ventral prostate lobe and improve histology of testis. Therefore, it may be helpful in the management of benign prostatic hyperplasia. Pumpkin seeds improve sexual stimulation and intromission and ejaculatory latency.

(Mohammadi *et al*, 2013).

DEXAMETHASONE

Glucocorticoids are the major steroid hormones secreted by adrenal gland that influence the activity of almost every cell in the body. They modulate the expression of approximately 10% of our genes and are essential for life but are also increasingly implicated in the pathogenesis of disease and produce many unwanted effects when given therapeutically. Glucocorticoids are strongly immunosuppressive and anti-inflammatory, which has made them one of the most frequently prescribed drugs worldwide. Glucocorticoid cause changes in plasma gonadotrophin level and their pituitary content and indirectly contribute to the inhibition of reproductive functions. (Khorsandi *et al*, 2008). As Glucocorticoids are widely used medicine and these agents affect testis homeostasis by decreasing the testosterone level. Glucocorticoids concentration precedes a decline in testosterone concentration in the male.(Orazizadeh *et al*, 2009).

REVIEW OF LITERATURE

Toxic effects of Antidepressants

- Safarinejad (2008) reported Sperm DNA damage and semen quality impairment after treatment with Selective Serotonin Reuptake Inhibitors detected using Semen Analysis and Sperm Chromatin Structure Assay and concluded that patients receiving SSRIs had impairment in all semen parameters and increased sperm DNA damage.

- Higgins *et al* (2010) reported antidepressant-associated sexual dysfunction:impact, effects, and treatment and concluded that SSRI group, cause sexual dysfunction in both men and women. The sexual problems reported are extensive, ranging from decreased sexual desire, decreased sexual excitement, diminished or delayed orgasm, to erection or delayed ejaculation problems.

- Tanrikut *et al* (2010) reported adverse effect of paroxetine on sperm and concluded that in men , Paroxetine induced abnormal sperm DNA fragmentation. The fertility potential of a substantial number of men was affected by the changes in sperm DNA integrity.

- Altuner *et al* (2013) reported the effect of Mirtazapine on Cisplatin-induced oxidative damage and infertility in rat ovaries and concluded that the MDA, MPO, NO groups and 8-OH Gua levels were higher in the cisplatin-treated groups than the controls.This was not observed in the mirtazapine and cisplatin groups.The oxidative stress induced by cisplatin in the rat ovary tissue causes infertility in the female rats.

- Erdemir *et al* (2014) reported the effect of Sertraline, Paroxetine, Fluoxetine and Escitalopram on testicular tissue and oxidative stress parameters in rats and concluded that Spermatogenesis was affected in all groups, but the most prominent effect was observed in the paroxetine group.

- Filho *et al* (2014) reported Fluoxetine induces changes in the testicle and testosterone in adult male rats exposed via placenta and lactation and concluded that there was reduction in the weight of testes, epididymis and

seminal gland, reduction in volume of seminiferous epithelium and leydig cells, reduction in seminiferous tubules length and daily sperm production and Increase in individual area of leydig cells and plasma testosterone.

- Jacobsen *et al* (2015) reported effect of Vortioxetine vs. Escitalopram on sexual functioning in adults with well-treated major depressive disorder experiencing SSRI-induced sexual dysfunction and concluded that switching antidepressant therapy to Vortioxetine may be beneficial for patients experiencing sexual dysfunction during antidepressant therapy with SSRIs.

- Montejo *et al* (2015) reported sexual side-effects of antidepressant and antipsychotic drugs and concluded that all antidepressants with serotonergic activity can cause mild to severe sexual dysfunction . Decreased libido and delayed orgasm frequently (>60%) or anorgasmia and arousal difficulties sometimes (30%). Noradrenergic, dopaminergic, or melatonergic antidepressants do not cause sexual dysfunction.

- Galal *et al* (2016) reported adverse effects of long-term administration of fluvoxamine on haematology, blood biochemistry and fertility in male albino rats: a possible effect of cessation and concluded the Induction of leukocytosis, lymphocytosis and monocytosis. fluvoxamine evoked hepatic, renal and cardiac dysfunction,risk of male infertility, which is indicated by its deleterious impacts on spermiogram and steroidogenesis hormones and induction of oxidative stress, apoptosis in testicular tissue.

- Atli *et al* (2017) reported Sertraline-induced reproductive toxicity in male rats: evaluation of possible underlying mechanisms and concluded that Sertraline caused an increase in sperm DNA damage and induced histopathological lesions in all groups treated with Sertraline. Decreased sperm count was accompanied by a significant increase in abnormal sperm morphology, DNA damage. Sertraline induced testicular toxicity in a dose-dependent manner.

- Ilgin *et al* (2017) reported Citalopram induces reproductive toxicity in male rats and concluded that in Citalopram-administered group sperm concentration was reduced, abnormal sperm morphology, sperm DNA damage were increased and testicular damage was accompanied by oxidative stress and hormonal changes.

- Bandegi *et al* (2018) reported effects of antidepressants on parameters, melondiadehyde and diphenyl-2-picryl-hydrazyl levels in mice spermatozoa and concluded that Amitriptyline consumption can weaken sperm parameters. Increased production of ROS and toxicity and Venlafaxine improved sperm parameters in mice and the lipid peroxidation did not change compared to the control group.

Pumpkin seeds as protective

- George *et al* (2012) reported antidepressive activity of processed Pumpkin (*Cucurbita maxima)* seeds on rats and observed decrease in duration of immobility time (sec) of all experimental rats compared with depressed control group in Force Swimming Test and Tail Suspension Test.
- Mohammadi *et al* (2013) reported effects of Herbal medicine on male infertility and concluded that medicinal herbs are used to treat sperm disorders, dysfunctioning of the libido, sexual asthenia and erection.
- Aghaie *et al* (2015) reported protective effect of Pumpkin seed extract on sperm characteristics, biochemical parameters and epididymal histology in adult male rats treated with Cyclophosphamide and concluded that Pumpkin seed extract increase the total antioxidant capacity (TAC) level, improve the histopathological changes such as vacuolisation, disorganisation and separation of epididymal epithelium.
- Ayaz *et al* (2015) reported that evaluation of the anthelmintic activity of pumpkin seeds (*Cucurbita maxima)* in mice naturally infected with *Aspiculuris tetraptera* and concluded that Pumpkin seed has high anthelmintic activity against nematodes as well as its continued use in traditional medicine for the treatment of helminthiasis.
- Chari *et al* (2018) reported an appraisal of Pumpkin Seed Extract in 1, 2 - Dimethylhydrazine induced Colon cancer in Wistar rats and concluded that there was difference in the aberrant crypt foci (ACF) number in all treatment groups compared to control and Dimethylhydrazine groups, decrease in the colon length/weight ratio, change in the colonic glutathione (GSH) and superoxide dismutase(SOD) levels when compared to control and Dimethylhydrazine control and the nitrite content was decreased in treatment group .

Dexamethasone as Positive control

- Khorsandi *et al* (2008) reported Dexamethasone effects on Fas Ligand expression in mouse testicular germ cells and concluded that there was increase in apoptotic indexes of germ cells and increase in FasL expression of testicular germ cells.
- Orazizadeh *et al* (2009) reported toxic effects of dexamethasone on mouse testicular germ cells and concluded that Dexamethasone induces histopathological alterations such as epithelial vacuolisation, atrophy and reduction in testicular spermatozoid, reduction in tubular diameter and epithelial height, poor spermatogenesis, apoptotic index of germ cells was increased.
- Khorsandi *et al* (2013) reported effect of curcumin on dexamethasone-induced testicular toxicity in mice and concluded that Bcl-2 expression was increased in the curcumin + dexamethasone group compared with dexamethasone-treated animals. Dexamethasone induced spermatogenesis defects including epithelial vacuolizations, sloughing of germ cells, reduction of seminiferous tubule diameter, reduction in the number of sperm heads and significant maturation arrest.

HYPOTHESIS

Ho: There is no significant role of Pumpkin seeds extract against reproductive toxicity in male *Swiss albino* mice caused by sub- chronic treatment of antidepressant drug; Escitalopram.

H1: There is a significant effect of Pumpkin seeds extract against reproductive toxicity in male *Swiss albino* mice caused by sub- chronic treatment of antidepressant drug; Escitalopram.

OBJECTIVES

To study the possible effect of pumpkin seeds against escitalopram antidepressant drug on male *Swiss albino* mice as a model with the following objectives-

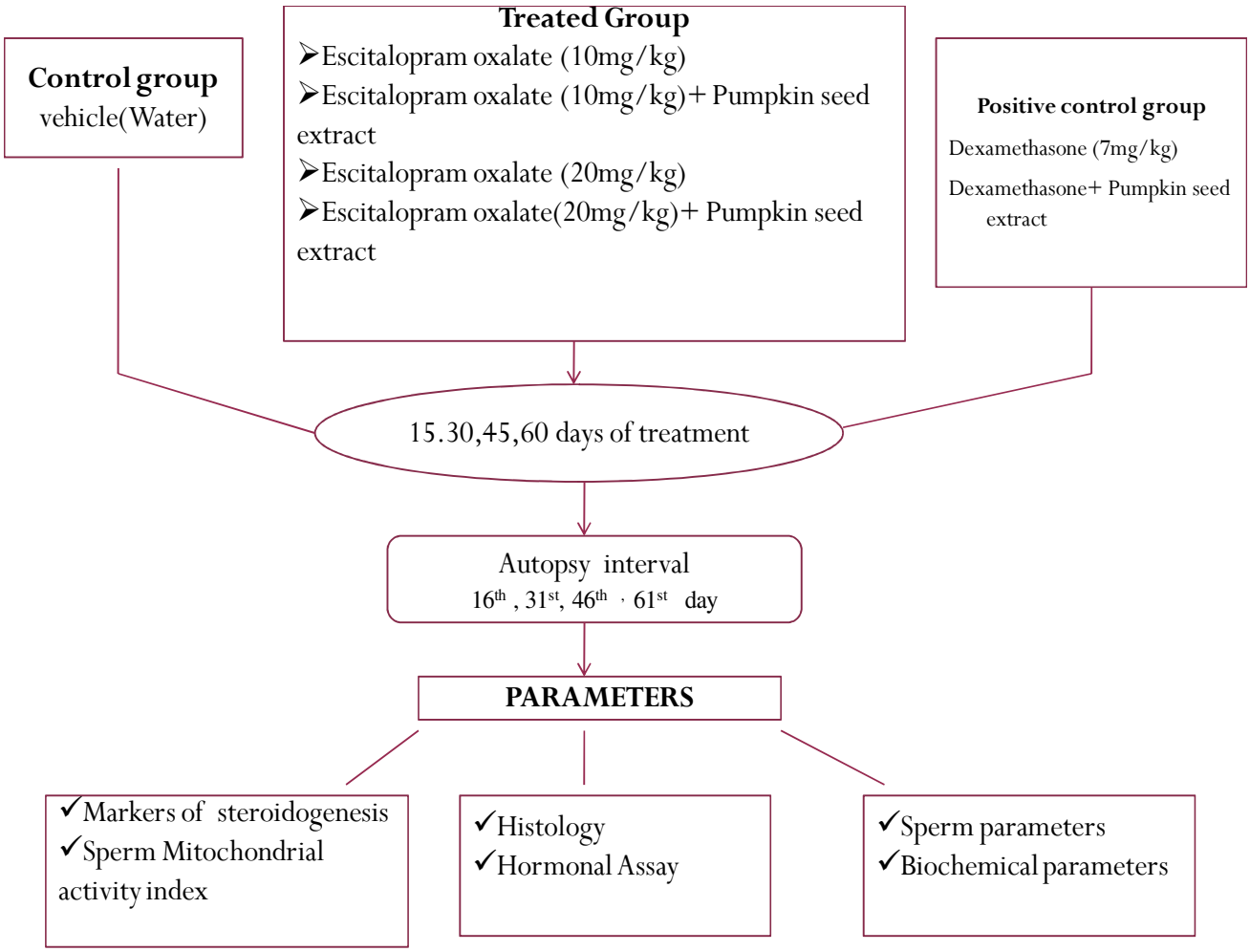
1. Observe the toxicity in reproductive organs caused by continuous use of escitalopram antidepressant drug (10mg/kg and 20mg/kg) after 15, 30, 45 and 60 days of treatment.
2. Compare the escitalopram drug induced toxicity with Dexamethasone (positive control) toxic group.
3. Study the effects of Pumpkin seeds extract against escitalopram drug in reproductive organs.

PLAN OF WORK

Experimental Animal -

Animal to be used for this study shall be adult male *Swiss albino* mouse (*Mus musculus*), approximately 6-8 weeks old and weighing between 25 to 30g .

Experimental protocol- Mice will be divided into eleven groups. In each group 12 animals will be used except in Group III, IV, VII, VIII which will have 3 animals.



- Group I– Control Group (Vehicle treated water)
- Group II- Escitalopram oxalate (10mg/kg)
- Group III- Escitalopram oxalate as in Group II for 30 days + Protective dose for next 15 days
- Group IV- Escitalopram oxalate as in Group II for 30 days + Protective dose for next 30 days
- Group V- Simultaneously administration of Escitalopram oxalate as in Group II + Protective dose for 15, 30,45, 60 days
- Group VI - Escitalopram oxalate (20mg/kg)
- Group VII- Escitalopram oxalate as in Group VI for 30 days + Protective dose for next 15 days
- Group VIII - Escitalopram oxalate as in Group VI for 30 days + Protective dose for next 30 days
- Group IX - Simultaneously administration of Escitalopram oxalate as in Group VI + Protective dose for 15, 30,45, 60 days
- Group X- Dexamethasone positive control
- Group XI- Dexamethasone + Protective dose

Total number of animals required- 96 animals

- **Mode of Administration** – Intubation through gavage pipe.
- **Duration of Experiment**- 2 months
- **Duration of Treatment**- 15, 30, 45, 60 days
- **Autopsy** will be done at 16^{th} , 31^{st}, 46^{th} , 61^{st} day
- **Dose-**
  Escitalopram oxalate – 10mg/kg and 20mg/kg body wt.

  Pumpkin seed extract as protective

  Dexamethasone as positive control - 7mg/kg body wt.

At the termination of experiment , animals will be sacrificed and the sample will be collected for further analysis or assessment.

Parameters which will be done by following methods-

A. Physical parameters-

- Body weight
- Organ weight

B. Sperm Analysis-

- Sperm Motility (Prasad *et al*,1972)
- Sperm count (Prasad *et al*,1972)
- Sperm viability (Talpot and Chacon,1981)
- Sperm deformities (Bruce *et al*,1974)

C. Serum hormone level-

- Testosterone hormone
- Leutinizing hormone
- Follicle-stimulating hormone

D. Histology-

- Testis
- Epididymis

E. Tissue (Testis) Biochemistry -

- Protein (Lowry *et al*,1951)
- Cholestrol (Zlatkis *et al*,1953)
- Fructose (in Seminal vesicle) (Foreman *et a1* 1973)
- Sialic acid (Warren *et al*,1959)
- Glycogen (Seifter *et al*,1950)
- Ascorbic acid (Roe and Kuether, 1943)

F. Markers of Steroidogenesis-

- 3β Hydroxysteroid dehydrogenase (Talalay *et al*, 1962)
- 17β Hydroxysteroid dehydrogenase (Talalay *et al*, 1962)

G. Sperm Mitochondrial activity index-

Nitroblue Tetrazolium test

H. Statistical Analysis-

The results will be evaluated statistically by using SPSS software.

SIGNIFICANCE

To find the effectiveness of Pumpkin seeds against the Escitalopram antidepressant drug toxicity, the seeds are commonly available in India.

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